

increased resistance to bacterial pathogens, increased resistance to viral pathogens, increased resistance to insects, modified flower size, modified flower number, modified flower pigmentation, modified flower shape, modified leaf number, modified leaf pigmentation, modified flower shape, modified seed number, a modified pattern of leaves and flowers, a modified distribution of leaves and flowers, modified stem length between nodes, modified root mass, increased drought tolerance, increased salt tolerance and increased antibiotic tolerance.

19. (Amended) A transgenic fruit-bearing plant comprising enhanced expression of a gene identified by the method of claim 1, wherein the gene is associated with a morphological characteristic selected from the group consisting of leaflet size, leaf size, leaf color, leaf shape, leaflet number, leaf number, internode length, plant height, floral organ characteristics and fruit characteristics.--

REMARKS

Claims 1-19 are pending for prosecution in this case. Claim 13 has been cancelled, leaving claims 1-12 and 14-19 pending for prosecution in the case.

Favorable consideration of the following comments relative to the outstanding rejections as they may apply to the present claims is respectfully requested for the reasons that follow.

I. Amendments.

A. To The Specification.

The specification has been amended as set forth above in response to the Examiner's objections based on informalities. Substitute paragraphs reflecting the amendments and a marked-up copy of the amended paragraphs of the specification are provided herewith. The amendments to the specification include no new matter.

B. To the claims

The claims have been amended as set forth above in response to the Examiner's objections based on informalities.

Claim 1 has been amended to reflect proper numbering of the steps, to replace the term "element which functions to enhance gene expression" with --an enhancer-- and to replace the term "termination element" with the term --"transcription termination element". Amended claim 1 finds support in claim 1, as filed.

Claims 2, 8, 9, 16 and 17 have been amended to more clearly recite the elements of the invention. Amended claims 2, 8, 9, 16 and 17 find support in claims 2, 8, 9, 16 and 17, as filed.

Claims 4-7, 10, 12, 14 and 15 have been amended to reflect proper claim dependencies.

Claims 14 and 15 have been amended to recite dwarf tomato plants. Amended claims 14 and 15 find support in claims 11, 12, 14 and 15, as filed.

Claims 13, 14 and 15 have been amended to replace the term "includes" with --is by--, consistent with the Examiner's suggestion.

Claim 19 has been amended to remove the word "claim". Claim 19 has been further amended to recite the morphological characteristics described in the specification at least on page As described in the specification at least on page 23, line 5 through page 23, line 4, which teaches that analysis of approximately 2000 plants generated by activation tagging has indicted the presence of a number of interesting morphological mutants, with exemplary phenotypes summarized in Table 1.

II. Objections To The Specification

With regard the objections to the specification based on the use of embedded hyperlinks, the specification has been amended as set forth above to remove the links.

With regard the objections to the specification based on the use of the trademark GLEAN™, Applicants have amended the relevant paragraph accordingly.

Applicants have noted the objections to the specification based on the use of brackets and have amended the relevant paragraphs accordingly, as set forth above.

III. Claim Rejections Under 35 U.S.C. §112, First Paragraph, Enablement.

Claims 18 and 19 stand rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and use the invention without undue experimentation.

A. Legal Standard for Enablement under 35 U.S.C. §112.

The first paragraph of 35 U.S.C. §112 requires that the specification of a patent enable any person skilled in the art to which it pertains to make and use the claimed invention. Enablement requires that the specification teach those in the art to make and use the invention without undue experimentation (e.g., *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir., 1991).

Nothing more than objective enablement is required, and therefore it is irrelevant whether this teaching is provided through broad terminology or illustrative examples (*In re Wright*, 999 F.2d 1557, 27 USPQ2d 1510 (Fed Cir. 1993). The enablement requirement is met

if the description enables any mode of making and using the claimed invention (*Engel Industries, Inc. v. Lockformer Co.*, 946 F.2d 1528, 20 USPQ2d 1300 (Fed. Cir. 1991).

An invention is enabled even though the disclosure may require some routine experimentation to practice the invention. *Hybritech Inc, V. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 U.S.P.Q. 81, 94 (Fed. Cir. 1986). The fact that the required experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation. *MLT. v A.B. Fortia*, 774 F.2d 1104, 227 U.S.P.Q. 428 (Fed. Cir. 1985).

Even in an unpredictable art, §112 does not require disclosure of a test of every species encompassed by the claims and the Examiner has the burden of showing that the disclosure entails undue experimentation. *In re Angstadt*, 537 F.2d 498, 190 U.S.P.Q. 214 (CCPA 1976).

B. Meeting the Legal standard

In accordance with the accepted standards of enablement set out above, an invention is enabled if one skilled in the art could make and use the claimed invention without undue experimentation.

1. In practicing the methods of the invention, as embodied in claim 18, one skilled in the art would have to make or provide the pSKI15, pAG 3201, pAG 3202 and pAG 4201 vectors.

With respect to claim 18, the Examiner says that the invention employs novel transformation vectors and that since they are essential to the claimed invention they must be obtainable by a repeatable method set forth in the specification or otherwise readily available to the public. Applicants submit that the specification provides ample guidance relative to a repeatable method for making the claimed vectors.

A schematic depiction of the pSKI15 and p3202 vectors is provided in Figs. 1 and 6, respectively. In addition, the specification states that "the key elements of pSKI15 are; (a) a pBstKS+ segment from the BluescriptTM plasmid, with an *E. coli* origin of replication (Stratagene), (b) the backbone from the RK2 plasmid, located between the left and right borders of the T-DNA, which contains the oriV and oriT regions responsible for stable replication in *Agrobacterium*; (c) a bialaphos resistance (BAR) gene encoding a phosphinothricin acetyltransferase enzyme; (d) a mannopine synthase (mas) promoter operatively linked to BAR gene, upstream thereof; (e) an octopine synthase (ocs) polyA termination element located downstream of the BAR gene, adjacent the left border of the plasmid, and (f) a tandem duplicated 35S enhancer element (3X)". (See page 14, lines 23-31 of the specification.)

Further, the specification states that "exemplary transformations are carried out using colonies of *Agrobacterium tumefaciens* strains EHA 105, EHA 101 or GV3101 containing a

binary plasmid, e.g., pAG3201(pSKI backbone with a 4x duplicated 35S enhancer and the nptII gene under the control of a CsVMV promoter), pAG3202 (pSKI backbone with a 4x duplicated 35S enhancer and the nptII gene under the control of an RE4 promoter, Fig. 6) or pAG4201 (pPZP-200 backbone with a 4x duplicated 35S enhancer and the nptII gene under the control of an RE4 promoter)." (See page 15, lines 1-7 of the specification.)

As the Examiner can see, the exemplary pAG3202 binary plasmid is based on the pSKI backbone, but includes the 4X 35S enhancer presented as SEQ ID NO: 4, instead of the tandem enhancer 35S enhancer element of pSKI15 and the nptII selectable marker under the control of an RE4 promoter instead of the bialaphos resistance (BAR) gene under the control of a mannopine synthase (mas) promoter, as provided in pSKI15.

Accordingly, the Examiner will appreciate that it is well within the knowledge of one of skill in the art to use routine techniques to modify a vector such as pSKI15 to include different or substitute components when the restriction sites and sequences are known. Hence, as set forth above, the description in the specification teaches one of skill in the art how to make the pSKI15, pAG 3201, pAG 3202 and pAG 4201 vectors without undue experimentation.

2. In practicing the methods of the invention, as embodied in amended claim 19, one skilled in the art would have to make or provide for a transgenic fruit-bearing plant comprising enhanced expression of a gene identified by carrying out the steps of:

(i) transforming cells of a plant with a plant cell expression vector having the components specific in claim 1 by introduction of an *Agrobacterium tumefaciens* vector into hypocotyl or shoot tip tissue in the absence of feeder cells, in a manner effective to express said selectable marker-encoding nucleotide sequence;

(ii) selecting plant cells which have been transformed by their ability to grow in the presence of an amount of selective agent that is toxic to non-transformed plant cells;

(iii) regenerating transformed plant cells to yield mature plants;

(iv) selecting plants having a desired trait; and

(v) identifying, isolating and characterizing genes the transcription of which was enhanced by said element which functions to enhance gene expression, wherein the enhanced gene expression is associated with a change in the level of: vitamins, a minerals, elements, amino acids, carbohydrates, lipids, nitrogenous bases, isoprenoids, phenylpropanoids or alkaloids.

With respect to amended claim 19, the Examiner acknowledges that the specification is enabling for a transgenic fruit-bearing plant comprising enhanced expression of a gene encoding SEQ ID NO: 27 or SEQ ID NO: 28 (paper 9, page 4). However, the Examiner argues

that the specification does not provide enablement for a transgenic fruit-bearing plant comprising enhanced expression of any gene. Amended claim 19 recites the components of the method that the Examiner agrees are enabled where the enhanced gene expression is associated with a morphological characteristic selected from the group consisting of leaflet size, leaf size, leaf color, leaf shape, leaflet number, leaf number, internode length, plant height, floral organ characteristics and fruit characteristics. The genes encoding SEQ ID NO: 27 and SEQ ID NO: 28 are associated with such a morphological characteristic, identified as a delayed flowering phenotype. (See page 23, line 5 and Example 2 of the specification.)

Given the teaching in the specification as to the components of the method that the Examiner agrees are enabled taken together with the example of identification of the morphological mutant designated " L23", Applicants submit that the specification teaches one of skill in the art how to make a transgenic fruit-bearing plant that exhibits enhanced expression of a gene the expression of which is associated with a morphological characteristic without undue experimentation.

In accordance with the accepted standards of enablement set out above, the invention is enabled because one skilled in the art could make and use the claimed invention without undue experimentation. Accordingly, Applicants submit that the pending claims comply with the enablement requirement of 35 U.S.C. §112, first paragraph.

V. The Rejections Under 35 U.S.C. §112, Second Paragraph

Claims 1-19 stand rejected under 35 U.S.C. § 112, second paragraph for failing to point out and distinctly claim the subject matter which Applicant regards as the invention.

Applicants have amended the claims as set forth above in order to expedite the prosecution of this case. Claims 1, 2, 4-7, 8-10, 12 and 14-17 have been amended as set forth above for purposes of clarity.

Each rejection is addressed individually, below.

Claim 1 stands rejected on the basis that the phrase "an element" which functions to enhance gene expression, is indefinite. Claim 1 has been amended to recite the term "an enhancer". Claim 1 also stands rejected on the basis that the phrase "termination element" is indefinite. Claim 1 has been amended to recite the term "transcription termination element", which Applicants submit one of skill in the art would understand to mean a sequence in DNA that signals the termination of transcription.

The Examiner argues that Claim 2 is indefinite in recitation of the term "in a manner effective to enhance". Applicants submit that the amended claim recites "wherein expression", obviating this basis for rejection.

Claims 4-7, 10, 12, 14 and 15 have been amended to reflect proper claim dependencies.

Claims 5, 7, 8 and 9 have been amended consistent with the Examiner's suggestion.

Claims 16 and 17 have been amended to reflect proper Markush format.

Applicants submit under 35 U.S.C. §112, second paragraph should therefore be withdrawn.

VI. Rejection under 35 U.S.C. §102(b)

The Examiner has rejected claims 1-4 and 191 under 35 U.S.C. §102(b) as anticipated by Hayashi *et al.*, (Science, 258:1350-1353, 1992), (Hayashi *et al.*)

A. The Invention

The invention is based on a method for identifying genes associated with a desired trait in a fruit-bearing plant by carrying out the steps of: transforming cells of a plant with a plant cell expression vector which has an *E. coli* origin of replication, an enhancer, a selectable marker-encoding nucleotide sequence operably linked to a promoter effective to express the selectable marker encoding sequence, a transcription termination element for said selectable marker-encoding nucleotide sequence, and a T-DNA sequence, wherein said transforming cells is by introduction of *Agrobacterium tumefaciens* into hypocotyl or shoot tip tissue derived from said plant in the absence of feeder cells, in a manner effective to express said selectable marker-encoding nucleotide sequence; selecting plant cells which have been transformed by their ability to grow in the presence of an amount of selective agent that is toxic to non-transformed plant cells; regenerating transformed plant cells to yield mature plants; (iv) selecting plants having a desired trait; and identifying, isolating and characterizing genes the transcription of which was enhanced by said element which functions to enhance gene expression.

B. The Prior Art

Hayashi *et al.*, describe a using a T-DNA tagging vector used to tag genes involved in the auxin independent division of tobacco (*Nicotiana tabacum*) protoplast-derived cells.

C. Analysis

1. Legal Standard for Anticipation

For a prior art reference to be anticipating under 35 U.S.C. §102(b), it must teach "each and every" element of the claimed invention. *In re Bond*, 15 USPQ2d 1566, 1567 (Fed. Cir. 1990).

2. Arguments.

The Examiner cites Hayashi *et al.* for disclosing a method or identifying genes associated with a edited trait in a tobacco plant using a method which the Examiner urges has all of the claim limitations of claims 1-4 and 19 of the instant application.

Applicants respectfully disagree.

The present claims are directed to a method for identifying genes associated with a desired trait in a fruit-bearing plant. Hayashi *et al.* does not describe fruit-bearing plants. Accordingly, the Hayashi *et al.* reference does not teach each and every element of the claimed invention and the rejection under 35 U.S.C. §102(b) should therefore be withdrawn.

VII. The Rejections Under 35 U.S.C. §103(a)

Claims 1-10 are rejected under 35 U.S.C. §103(a) as unpatentable over Hayashi *et al.* The examiner has taken the position that it would be *prima facie* obvious to one of skill in the art to modify the method of Hayashi *et al.*, to use other promoters, enhancers, selectable markers and transformation vectors based on the argument that promoters, enhancers, selectable markers and transformation vectors are functional equivalents. Applicants respectfully traverse.

A. The Invention. The invention is described above.

B. The Prior Art

Hayashi *et al.* is described above.

C. Analysis

1. Legal Standard For Nonobviousness

With respect to the Examiner's rejection of the claims, the PTO has the burden of establishing a case of *prima facie* obviousness.

A proper analysis under §103 requires, *inter alia*, consideration of two factors: (1) whether the prior art would have suggested to those of ordinary skill in the art that they should make the claimed composition or device, or carry out the claimed process; and (2) whether the prior art would have also revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of success....Both the suggestion and the reasonable expectation of success must be founded in the prior art, not in the applicant's disclosure. See MPEP §2142, citing *In re Vaeck*, 20 USPQ2d 1438, Fed. Cir. 1991.

Thus, for a combination of references to render a claimed invention obvious under 35 U.S.C. § 103, that combination must provide not only a suggestion of the present invention, but also a reasonable expectation of success in reaching that invention.

2. Arguments

The Examiner argues that "given the success of Hayashi in identifying a desired trait and isolating genes using the taught method one of ordinary skill in the art would have reasonable expectation of success in using other promoters, transformation methods and plants to identify desirable traits."

Applicants respectfully disagree and submit that one of skill in the art relying on Hayashi et al. would not have reasonable expectation of success in practicing the invention as claimed for the following reasons:

A team of researchers at the Cologne institute, along with colleagues from other European labs, could not reproduce the results published in Science, EMBO Journal, the Proceedings of the National Academy of Sciences (PNAS), Trends in Plant Science, and Plant Journal, based on the experiments of Hayashi et al. as a result, Hayashi et al. has been retracted, as stated in EMBO J, 18 (10)2908, 1999, wherein it states, "we hereby retract officially the results regarding auxin independent division of tobacco protoplast-derived cells in our papers mentioned above." Hayashi *et al.*, Science, 258:1350-1353, 1992, is one of the paper mentioned above. (A copy of retraction is included herewith.)

In addition, at the time the application was filed, activation tagging had not been shown to be practical in fruit-bearing plants, an element of the present claims.

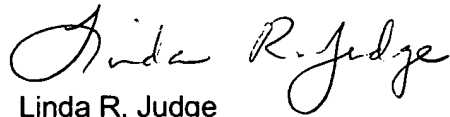
Further, the hypocotyl or shoot tip transformation method which does not require the use of feeder cells or nurse cultures and is employed to introduce *Agrobacterium* vectors into plant cells is a novel and nonobvious feature of the current invention..

As stated in Beckman Instruments v. LKB Produkter AB, 892 F.2d 1547, 1551, 13 USPQ2d 1301, 1304 (Fed. Cir. 1989), "even if a reference discloses an inoperative device, it is prior art for all that it teaches. Hence, taken together, the Hayashi et al. reference and the retraction thereof teach against a reasonable expectation of success in practicing the invention. In addition, Hayashi does not teach or suggest activation tagging in fruit-bearing plants or the novel transformation methods provided by the present invention.

Accordingly, the examiner has not made a case of *prima facie* obviousness and the rejection under 35 U.S.C. §103(a) should therefore be withdrawn

If in the opinion of the Examiner a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 838-4404.

Respectfully submitted,



Linda R. Judge

Registration No. 42,702

Date: *August 6, 2001*

Correspondence Address:

Customer No. 22918

09/522,334

Atty. Docket No. 4257-00218.30

Amendments to specification filed August 6, 2001

In the specification, the paragraph beginning at line 20 on page 14 of the application as filed, has been replaced with a new paragraph revised as follows:

--An exemplary vector for use in the methods of the invention is the pSKI15 plasmid. (See, [<http://www.biosun.asalk.edu/LABS/pbio-w/index.html>]; Hayashi *et al.*, Science 258: 1350-1353, 1992; Walden *et al.*, Plant Mol Biol 26:1521-1528).--

The paragraph beginning at line 38 on page 20 of the application as filed, has been replaced with paragraph revised as follows:

--The identified genomic insertion sequence is used to do NCBI BLASTTM similarity search [using the interface provided at <http://www.ncbi.nlm.nih.gov/BLAST/>] by carrying out a basic BLASTN search of non-redundant nucleic acid sequence databases through NCBI, using the search capabilities of the NIH website. The BLAST search results indicate the presence or absence of related sequences which have been deposited in the public databases that are searched, as of the date of the search.—

The paragraph beginning at line 42 on page 20 of the application as filed, has been replaced with paragraph revised as follows:

--In general, the largest rescued plasmid is used to design new primers to sequence the full-length genomic insertion. Such primers may be designed using a computer program, for example, the Primer3 program found on the Internet using the website from the Whitehead Institute for Biomedical Research/MIT [at <http://www.genome.wi.mit.edu/cgi-bin/primre/primer3/www.cgi/>].--

The paragraph beginning at line 6 on page 254 of the application as filed, has been replaced with paragraph revised as follows:

The sequencing resulted in identification of a 4437 bp DNA sequence (Figures 9A-9B, SEQ ID NO:26). A Basic BLASTN search [(<http://www.ncbi.nlm.nih.gov/BLAST/>)] of non-redundant nucleic acid sequence databases, conducted on Feb. 29, 2000, through NCBI [(<http://www.ncbi.nlm.nih.gov/index.html>)] using the search capabilities of the NIH website, with the nucleotide sequence presented in Figures 9A-9B revealed no significant sequence identity between sequences available in GenBank and nucleic acids 1-4437 of the SEQ ID NO:26.

The paragraph beginning at line 12 on page 25 of the application as filed, has been replaced with paragraph revised as follows:

Two open reading frames were predicted in the rescued sequence using the GENESCAN computer program, [found at "MIT <http://CCR-081.mit.edu/GENESCAN>"] which may be found on the Internet using the GENSCAN Web Server of the MIT website, indicating the presence of genes which encode polypeptides of about 124 and 85 amino acids, respectively (Fig. 10A, SEQ ID NO:27 and Fig. 10B, SEQ ID NO:28, respectively).

The paragraph beginning at line 5 on page 14 of the application as filed, has been replaced with paragraph revised as follows:

--In further embodiments, the methods of the invention are carried out using a vector which includes an herbicide resistance gene, conferring resistance to glyphosate-containing herbicides. Glyphosate refers to N-phosphonomethyl glycine, in either its acidic or anionic forms. Herbicides containing this active ingredient include "ROUNDUP" and "GLEANTM". Exemplary genes for imparting glyphosate resistance include an EPSP synthase gene (5-enolpyruvyl-3-phosphoshikimate synthase) (Delannay, *et al.*, 1995; Tinius, *et al.*, 1995), or an acetolactate synthase gene (Yao, *et al.*, 1995).--

The paragraph beginning at page 2, line 1 of the application as filed, has been replaced with paragraph revised as follows:

Insertional mutagenic techniques have also been used to generate random modifications of native plant genes. For example, the T-DNA insertion technique, termed "T-DNA tagging" or "activation tagging" has been used to develop large numbers of transformed plant lines, *e.g.*, in *Arabidopsis* (Christensen, S., *et al.*, 9th INTL. CONF. ON ARABIDOPSIS RES. June 24-28, 1998, p 165, Univ. Of Wis.), as well as in the legume, *Medicago truncatula* (Kardailsky, I, *et al.*, 9th INTL. CONF. ON ARABIDOPSIS RES. June 24-28, 1998, p.187-188, Univ. Of Wis.). In this technique, seeds are transformed with the Ti plasmid from *Agrobacterium tumefaciens* which is inserted randomly into the plant genome. [See, *e.g.*, Feldmann, KA, *Plant J.* 1:71, 1991; Hayashi H *et al.*, *Science* 258 (5086):1350-3, 1992; Walden, R., *et al.*, *Plant Molecular Biology*, 26:1521, 1994]. The isolation of the floral inducer FLOWERING LOCUS T (FT), which acts in parallel with the meristem-identity gene LEAFY (LFY) to induce flowering in *Arabidopsis* using activation tagging has recently been described (Kardailsky I *et al.*, *Science* 286(5446):1962-5, 1999).

The paragraph beginning at page 7, line 3 of the application as filed, has been replaced with paragraph revised as follows:

As used herein, the term "sequence identity" means nucleic acid or amino acid sequence identity in two or more aligned sequences, aligned using a sequence alignment program. Sequence searches are preferably carried out using the BLASTN program when evaluating the of a given nucleic acid sequence relative to nucleic acid sequences in the GenBank DNA Sequences and other public databases. The BLASTX program is preferred for

searching nucleic acid sequences which have been translated in all reading frames against amino acid sequences in the GenBank Protein Sequences and other public databases. Both BLASTN and BLASTX are run using default parameters of an open gap penalty of 11.0, and an extended gap penalty of 1.0, and utilize the BLOSUM-62 matrix. {See, Altschul, *et al.*, Nucl. Acids Res. 25(17) 3389-3402 (1997).}

The paragraph beginning at page 8, line 38 of the application as filed, has been replaced with paragraph revised as follows:

A fraction of the plants in which the expression of native genes is enhanced will exhibit desired traits. The plants which exhibit such desired traits are selected and the plant genomic DNA flanking the insertion site of the enhancer sequence of the activation tagging nucleic acid construct identified and characterized. Techniques routinely employed by those of skill in the art for identification and isolation of genes of interest are plasmid rescue [(Behringer, F.J and Medford, J.I., *Plant Mol. Biol. Reporter* 10: 190-198, (1992)], and genome walking (e.g., GenomeWalker™ from Clontech, Palo Alto, CA).

The paragraph beginning at page 9, line 6 of the application as filed, has been replaced with paragraph revised as follows:

In some cases, inverse PCR may be used to isolate DNA adjacent known sequence in genomic DNA, by use of oligonucleotide primers complementary to one end of a known sequence that prime in opposite directions, and have a particular restriction enzyme site between them, e.g., the left or right border Ti sequences. In the method, chromosomal DNA is digested with a restriction endonuclease and ligated into a circularized DNA molecule. The resulting population of ligated molecules is comprised of a complex mixture of chromosomal DNA and chromosomal-vector DNA hybrids. The plasmid derived region of the hybrid molecules provides the downstream priming site for PCR amplification. The upstream primer may be specific for the vector, or a gene-specific primer. {See, e.g., Novak, J and Novak, L, *Promega Notes Magazine* Number 61:27, 1997}.

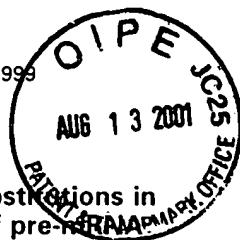
The paragraph beginning at page 10, line 6 of the application as filed, has been replaced with paragraph revised as follows:

Random expression of native genes may also be achieved by introduction of a nucleic acid construct comprising a transposon into the genome of interest. Exemplary transposons such as Ac, Ds, Mu or Spm are elements which can insert themselves into genes and cause unstable mutations. The mutations are unstable due to subsequent excision of the transposon from the mutant locus during plant or seed development. (See, e.g., Doring, H. P. and Starlinger (1986), *Ann. Rev. Genet.* 20:175-200; Federoff, N. (1989), "Maize Transposable Elements" in *Mobile DNA*. Wowe, M. M. and Berg, D. E., eds., Amer. Soc. Microbiol., Wash.,

D.C., pp. 377-411.) An exemplary transposon-tagging strategy used to identify a semi-dominant mutation affecting plant height, hypocotyl elongation, and fertility has been described. [See, Wilson K. *et al.*, *Plant Cell* 8(4):659-71, 1996.]

The paragraph beginning at page 11, line 21 of the application as filed, has been replaced with paragraph revised as follows:

Binary Ti-based vector systems are used to transfer and confirm the association enhanced expression of a given gene with the modified trait or phenotype of the plant. Appropriate vectors for this aspect of the invention include plasmids containing at least one T-DNA border sequence (left, right or both), restriction endonuclease sites for the addition of one or more heterologous nucleic acid sequences [adjacent flanking T-DNA border sequence(s)], a heterologous nucleic acid sequence (*i.e.*, the coding sequence of identified and isolated genes), operably linked to appropriate regulatory sequences and to the directional T-DNA border sequences, a selectable marker which is functional in plant cells, a heterologous Ti-plasmid promoter, an *E. coli* origin of replication.



Retraction

Site-specific deoxynucleotide substitutions in yeast U6 snRNA block splicing of pre-mRNA *in vitro*

C.H.Kim, D.E.Ryan, T.Marciniec and J.Abelson

The EMBO Journal, 16, 2119–2129, 1997

We (the authors) wish to report the following correction regarding our studies of deoxynucleotide-substituted U6 snRNAs in yeast spliceosomes. In our original work, we tested 50 site-specific deoxynucleotide substitutions in U6 RNA for their effects on splicing. Of these, only four specific deoxynucleotides blocked splicing and did so reproducibly. Recently, we repeated these experiments using the original stocks of the deoxy-substituted pieces, and we observed that splicing was not blocked or diminished relative to controls. Multiple attempts to reproduce our published results have failed. However, some of the original conditions cannot be replicated. The U6 RNA is synthesized *in vitro* in these experiments via ligation of four or five synthetic oligonucleotide pieces. Although we still have stocks of the original yeast extract and deoxy-substituted oligonucleotides, the original stocks of the flanking pieces of U6 RNA had been depleted. Hence, we are unable to duplicate the reported experiments exactly. Although we have tested various parameters, including various extracts, preparations of the U6 RNA pieces, and U6 reconstitution conditions, we are unable to find conditions under which the four deoxy substituents in question have any deleterious effect on splicing. In any case, the recent observations of normal splicing for these four substituents mean that they do not block splicing generally. Though we are not now able to reproduce the reported observations for the four deoxy substitutions, they may well have a deleterious effect on splicing under conditions not yet understood. We are left with the revised conclusion that synthetic U6 RNAs substituted with a single deoxynucleotide at any of the 50 positions tested (39–88 in yeast U6) are able to reconstitute splicing activity under standard conditions *in vitro*.

Retraction

Auxin inducibility and developmental expression of axi 1: a gene directing auxin independent growth in tobacco protoplasts

R.Walden, H.Hayashi, H.Lubenow, I.Czaja and J.Schell

The EMBO Journal, 13, 4729–4736, 1994

A plant cation–chloride co-transporter promoting auxin-independent tobacco protoplast division

H.Harling, I.Czaja, J.Schell and R.Walden

The EMBO Journal, 16, 5855–5866, 1997

In a recent article in the journal *Science* volume 283, pages 1987–1989 (1999) it is said that I (Schell) had no plans to publish retractions of the papers in the journals in which they had originally appeared. In fact, I wanted to stress the point that the first responsibility the collaborating colleagues in and outside the Institute and I had felt was to publish our results showing that the previously published data could not be reproduced by another, more objective method. Therefore, the members of the investigating team decided to publish all further data re-evaluating this fraud as a regular scientific paper in *The Plant Journal*. After peer review and acceptance of the paper, it was agreed with the Editor-in-Chief of *The Plant Journal*, Professor Diana Bowles, that after publication short correction statements should be sent to individual journals, which could refer to this paper for full details of new experiments confirming the irreproducibility of the protoplast assays in question (Schell *et al.*, 1999). Since the paper has now appeared, we hereby retract officially the results regarding auxin independent division of tobacco protoplast-derived cells in our papers mentioned above.

Reference

Schell,J., Bisseling,T., Dulz,M., Franssen,H., Fritze,K., John,M., Kleinow,T., Leßnick,A., Miklashevichs,E., Pawlowski,K., Rohrig,H., van de Sande,K., Schmidt,J., Steinbüß,H.-H. and Stoll,M. (1999) Re-evaluation of phytohormone-independent division of tobacco protoplast-derived cells. *Plant J.*, 17, 461–466.

- RNA-directed RNA polymerase-specific cDNA clone from tomato, *Plant Cell* 10, 2087–2101
- 8 Kennerdell, J.R. and Carthew, R.W. (1998) Use of dsRNA-mediated genetic interference to demonstrate that *frizzled* and *frizzled 2* act in the wingless pathway. *Cell* 95, 1017–1026
- 9 Montgomery, M.K., Xu, S. and Fire, A. (1998) RNA as a target of double-stranded RNA mediated genetic interference in *Caenorhabditis*

- elegans*. *Proc. Natl. Acad. Sci. U. S. A.* 95, 15502–15507
- 10 Ngô, H. et al. (1998) Double-stranded RNA induces mRNA degradation in *Trypanosoma brucei*. *Proc. Natl. Acad. Sci. U. S. A.* 95, 14687–14692
- 11 Waterhouse, P., Graham, M. and Wang, M.B. (1998) Virus resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA. *Proc.*

- Natl. Acad. Sci. U. S. A.* 95, 13959–13964
- 12 Tabler, M. and Sänger, H.L. (1984) Cloned single- and double-stranded DNA copies of potato spindle tuber viroid (PSTV) RNA and co-inoculated subgenomic DNA fragments are infectious, *EMBO J.* 3, 3055–3062
- 13 Jorgensen, R.A. et al. (1998) An RNA-based information superhighway in plants. *Science* 279, 1486–1487

Stephen Hales and the cohesion theory

In science, theories can be born more than once, as the mendelian laws have shown. In this context, the cohesion theory of water movement in plants has been variously ascribed to Josef Böhm¹, Henry H. Dixon and John Joly², and Eugen Askenasy³. However, all of the elements of this theory were first described in 1727 by the English clergyman Stephen Hales in his book *Vegetable Statics*⁴. Unfortunately, Hales' ideas were not understood at the time, so his findings failed to influence the debate on water transport in plants in the 19th century.

Hales' insight appears to have been influenced by his relations with Isaac Newton. Although Newton left Cambridge in 1696 when Hales entered, they met in 1718 when Hales was elected a fellow of the Royal Society. As chairman of the society, Newton gave Hales' book his imprimatur.

The most relevant section of *Vegetable Statics* is exp.33. After citing Newton's *Optics* (2nd edn, 1717; query 31, in which mercury is lifted 60–70 inches in a barometer tube by cohesion compared with a water pillar of over 60 feet) Hales wrote, 'And by the same principle it is, that we see, in the preceding experiments, plants imbibe moisture so vigorously up their fine capillary vessels; which moisture, as it is carried off in perspiration, (by the action of warmth) thereby gives the sap-vessels liberty to be almost continually attracting of fresh supplies; which they could not do, if they were full saturate with moisture: for without perspiration the sap must necessary stagnate, notwithstanding the sap-vessels are so curiously adapted by their exceeding fineness, to raise a sap to great heights, in a reciprocal proportion to their very minute diameters.' Hales' discussion of water conduction in plants is based on sound experiments, such as his measurements of tensions in transpiring branches. He noted

that tensions of up to 12 inches of mercury are not the full tension as air is sucked out of a branch simultaneously. He also produced an early dendrometer, and measured imbibition forces in peas.

As with so many original arguments, Hales' sounds superficial on first reading, although there can be no doubt of the mechanism involved. Hales also failed to name his theory. Thus, four editions and translations of his book were insufficient to connect the name of this versatile clergyman with the cohesion theory.

References

- 1 Böhm, J. (1893) Capillarität und Saftsteigen, *Ber. Dtsch. Bot. Ges.* 11, 203–212
- 2 Dixon, H.H. and Joly, J. (1894) On the ascent of sap, *Ann. Bot.* 8, 468–470
- 3 Askenasy, E. (1895) Über das Saftsteigen, *Verhandl. d. Heidelb. Naturhist.-Med. Vereins N. Serie V*, 325–345
- 4 Hales, S. (1727) *Vegetable Statics*, W. & J. Innys and T. Woodward, London. Reprint 1969 MacDonald, London and Elsevier, New York

Franz Floto

Dept of Plant Physiology, University of Copenhagen, Oe. Farimagsgade 2A, DK-1353 Copenhagen K, Denmark (tel +45 35322124; fax +45 35322125; e-mail floto@pfa.molbio.ku.dk)

Letters to Trends in Plant Science

Correspondence in Trends in Plant Science may address topics raised in very recent issues of the magazine, or other matters of general current interest to plant scientists. Letters should be sent, together with a disk copy, to the Editor (or e-mail your text to plants@elsevier.co.uk).

Retraction by Jeff Schell

In a recent article in *Science* (1999) 238, 1987–1989 it is said that I had no plans to publish retractions of the papers in the journals in which they originally appeared. In fact, I wanted to stress the point that the first responsibility the collaborating colleagues in- and outside the Max-Planck Institute and I had felt was to publish our results showing that the previously published data could not be reproduced by another, more objective, method. Therefore, the members of the investigating team decided to publish all further data re-evaluating this fraud as a regular scientific paper in *The Plant Journal*. After peer-review and acceptance of the paper it was agreed with the Editor-in-Chief, Prof. Dianna Bowles, that after publication short correction statements should be sent to individual journals, which could refer to the paper for full details of the new experiments, confirming the irreproducibility of the protoplast assays in question. As the paper has now appeared, we hereby retract officially the results regarding phytohormone effects on division of tobacco protoplast-derived cells in our papers:

- Hayashi et al. (1992) *Science* 258, 1350–1353
- Walden et al. (1994) *EMBO J.* 13, 4729–4736
- Röhrig et al. (1995) *Science* 269, 841–843
- Miklashevichs et al. (1996) *Trends Plant Sci.* 1, 411
- Röhrig et al. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 13389–13392
- Van de Sande et al. (1996) *Science* 273, 370–373
- Harling et al. (1997) *EMBO J.* 16, 5855–5866
- Miklashevichs et al. (1997) *Plant J.* 12, 489–498
- Please refer to our new results published in Schell et al. (1999) *Plant J.* 17, 461–466.

Jeff Schell

Max-Planck-Institut für Züchtungsforschung, Carl-von-Linné-Weg 10, 50829 Köln, Germany (e-mail schell@mpiz-koeln.mpg.de)